Montana Department of
ENVIRONMENTAL QUALITY

Water Quality Planning Bureau

Sample Collection and Laboratory Analysis of Chlorophyll-a

Standard Operation Procedure

Approvals:

(Signature on file)

Prepared by: Mark Bostrom, QA Officer, Water Quality Planning Bureau Date

(Signature on file)

Michael Suplee, PhD., Water Quality Specialist, WQ Standards Section Date

(Signature on file)

Rosie Sada, Section Supervisor, Water Quality Monitoring Section Date

Table of Contents

Chlorophyll-a

Chlorophyll-a is measured as a means of estimating algae (periphyton or phytoplankton) biomass in a body of water. It is usually expressed as either attached biomass for periphyton (mg/m^2), or as a concentration for plankton species (µg/L). Heavy growths of algae generally indicate inferior water quality.

Excess algae growth may clog water filters and irrigation equipment, cause taste and odor problems in water supplies, reduce instream dissolved oxygen levels, interfere with fish spawning, degrade macroinvertebrate habitat, trap sediment, deflect stream flows, and impair the overall aesthetics of a stream.

1. Scope and Applicability

This method is developed for use in water quality assessments[1] to determine beneficial use support of primary contact recreation and as an additional line of evidence supporting impairment determinations for aquatic life and fisheries uses due to excessive nutrients.

The sampling methods described herein are primarily used in wadeable streams and rivers. The phytoplankton sampling procedure may be used in low-flow conditions (disconnected series of pools) or in lakes and reservoirs.

These sampling methods are designed to produce a quantitative measure of algae growth by relating the total mass of chlorophyll-a pigment to a known area or volume.

1.1. Index period

Periphyton growth is controlled by season, nutrient concentrations, velocity of the current, days of accrual, grazing, shading, water temperature, and other factors. Because of this, an index period is necessary to provide a consistent period of time when stable flows have been achieved and diversity and standing crop have peaked. The summer period of June 21 to September 21 is selected as the period representing the maximum growth potential under these conditions in western Montana (mountainous region). A recent study[2] performed by DEQ in the northern glaciated plains (Hi-line study) used an index period of May – September to represent the period of maximum growth.

Samples collected for a water quality investigation (during the index period) are compared to standards or recommended concentrations expressed as either maximum concentrations, or mean values for the index period.

In the absence of established numeric standard or recommendations based upon a scientific study, reference conditions may be used to assess beneficial use support provided they are comparable to the investigation samples in terms of time sampled and methods used. It may be necessary to sample outside the index period to coincide with flows in ephemeral or dewatered streams, or to track seasonal changes. In these cases, reference conditions for the alternate period must be established.

---

[1]SOP WQPBWQM-001 – Montana Department of Environmental Quality, Water Quality Assessment Process and Methods, 06/2004.

[2] Wadeable Streams of Montana's Hi-line Region: An Analysis of Their Nature and Condition, with an Emphasis on Factors Affecting Aquatic Plant Communities AND Recommendations to Prevent Nuisance Algae Conditions. Suplee, M. W, Montana Department of Environmental Quality, May 2004.

When monitoring trends, minimize variability by sampling on or about the same date each year. High flows and turbid waters should be avoided because they limit access and obscure visibility of the stream bottom. Sampling should be delayed for at least two weeks following high, bottom-scouring stream flows to allow for recolonization by algae and succession to a mature periphyton community.

1.2. Site Locations and Extent

Selection of sampling *locations* depends largely on the objectives of the water quality study. Criteria for locating sites should be established in the sampling study design (pre-fieldwork) and sampling locations selected. If sampling locations are to be determined in the field, a field guide describing rationale for locating sites should be prepared to maintain integrity of the study design. This is very important when multiple sampling crews are selecting locations based on professional judgment.

The *extent* of individual sampling sites depends on the type of stream to be assessed. For high-gradient streams the extent of the sampling site is typically a single riffle. For low-gradient streams, the extent is typically one meander length or about 20 bank full channel widths.

1.3. Water Quality Standards and Reference Condition

1.3.1. Western Montana

The Administrative Rules of Montana (ARM 75-5-631) list numeric Chlorophyll-a *standards* for the Clark Fork River. These are:

Periphyton
Single sample maximum 150 mg/m^2
Mean summer concentration (June 21 to Sept. 21) 100 mg/m^2

Waterbodies other than the Clark Fork that are located in the western (mountainous) region of Montana should use a reference condition (Section 1.3.3) as the first option for determining beneficial use support. In the absence of adequate reference conditions, the water quality assessment may <u>*consider*</u> chlorophyll-a values developed by the British Columbia Ministry of Water, Land and Air Protection. *(Values used by British Columbia are not official water quality standards of Montana)*

Periphyton
Aquatic Life streams (max) 100 mg/m^2
Recreation streams (max) 50 mg/m^2

1.3.2. Northern Glaciated Plains

The DEQ Hi-Line study (footnoted earlier) of chlorophyll-a values in the northern glaciated plains of eastern Montana was completed by DEQ's Water Quality Standard section in May 2004. This study recommends the following guidance for chlorophyll-a values in the northern glaciated plains (*These values have not been adopted as a numeric standard through the administrative rule making process as of the publication of this SOP*):

Periphyton
Single sample maximum 110 mg/m^2
Mean summer concentration (May – Sept.) 65 mg/m^2

Phytoplankton
Single sample maximum 20 ug/L
Mean summer concentration (May – Sept.) 15 ug/L

These recommendations are generally adequate for making beneficial use support determinations in the northern glaciated plains region. However, the full text of the study should be reviewed to determine if reference conditions used within the Hi-line allow for a more specific reference condition comparison as well.

1.3.3. Reference Conditions

A reference site should be comparable to the study site in terms of depth, gradient, canopy cover (shading), substrate, and other physical features. Reference sites may be within the same watershed or region (external reference), on other, minimally disturbed sites on the stream being studied (internal reference), or based on historical data or information (historical reference). For long-term monitoring, reference site should be afforded sufficient protection as to maintain existing water quality throughout the duration of the study. It should be located on the same waterbody as the study site or in a local tributary to the study stream with the same stream order.

1.4. Sampling Quality Control

This SOP does not attempt to designate the quality control samples needed to understand reproducibility, representativeness, or bias associated with these sampling techniques. The appropriate Quality Control samples to assess field collection activities must be designated in the project planning documents (QAPP, SAP).

2. Sample Collection Methods

Periphyton standing crop may be quantified by measuring the amount of accrual on natural substrates at the study site. The sampling of artificial substrates is not recommended.

There are four methods for collecting attached algae (periphyton – rock, hoop, core, template) from streams and rivers and one method for collecting phytoplankton algae from pools or lakes. These are used individually or in combination to characterize a site's total chlorophyll-a concentration. The substrate at the sampling site will largely determine the best technique for collecting the sample.

The various collection techniques may not be directly comparability to another (e.g., some methods sample mainly diatom algae growth, while other methods capture primarily filamentous algae). The sampling design may have to consider collecting reference samples (using the same techniques as the study samples) in order to provide a comparable reference point.

2.1. Rock sampling method

The rock method is used for sampling substrate dominated by small boulders, gravel and cobble.

2.1.1. Method Summary

The rock collection method is the collection of a minimum of six rocks representing the dominant substrate along a transect line. The six rock samples are sent to a laboratory for extraction and chlorophyll-a analysis.

2.1.2. Sampling Equipment

- Waders
- Large freezer storage bags
- Aluminum foil
- Cooler
- Dry ice or regular ice

2.1.3. Sample Collection

Sampling site is across an undisturbed transect of the waterbody.

Collect a minimum of six (submerged) rock samples across the stream transect at even intervals. The six rocks should include the various sizes of rocks available but not be too small (smaller than a golf ball) or too large (larger than a softball).

2.1.4. Sample Handling & Labeling

Place the six rocks into large freezer Ziploc bags; place a label identifying the sample on the outside of the Ziploc bag.

Wrap the bag with aluminum foil leaving no space for light to enter. Place this wrapped bag into another large Ziploc bag and hand write the Activity ID on the outer bag with a **SHARPIE.**

Immediately store the sample on ice and away from light. Samples should be sent to the laboratory as soon as possible for chlorophyll-*a* analysis (monochromatic, corrected for pheophytin-a).

2.2. Template sampling method

The template method is an alternative to the rock method, and is also used for sampling transects with substrate dominated by small boulders, cobble and gravel. This method requires the sampler to pay closer attention to the density of material being collected, in order to assure that sufficient material is sampled to achieve a detectable chlorophyll level.

2.2.1. Method Summary

A template of know surface area (usually in the range of 12-25 cm^2) is placed on rocks with representative algae densities along a transect line. The area within the template is scraped into a container and sent to a laboratory for extraction and measurement of chlorophyll a. The template can be made, for example, of a cut-off piece of PVC pipe of known diameter.

2.2.2. Sampling Equipment

- Waders
- 50 cc centrifuge tube
- Knife for scraping rock
- Tooth brush for brushing rock
- Shallow plastic pan to hold rock
- Hand pump vacuum with tubing (*optional*)
- Nalgene filtering unit (*optional*)
- GFF filters (0.45 um) (*optional*)
- Tweezers or forceps (*optional*)
- Cooler with ice or dry ice

2.2.3. Sample Collection

A representative rock is placed in the shallow pan and the template placed over the upper (light-facing) surface of the rock. All of the growing material within the template is scraped and placed in the centrifuge tube.

In certain cases the volume of algal material on the rock surface is small, therefore it is better to scrub the rock surface with a toothbrush and then rinse the rock surface and the toothbrush in to the pan with a small volume of stream water (note: if stream water has a noticeable green tint use tap or distilled water). In such cases, a second template or (even third) from the same or a different rock may be collected and composited with the first to assure enough material is collected to achieve adequate chlorophyll detection.

As an option in cases where the rock surfaces were very clean and the toothbrush has been used, the algae material rinsed into the pan may be field filtered onto a GF/F filter and the filter placed in the centrifuge tube. Refer to Section 2.5.3 below for proper use of the Nalgene filtering unit.

2.2.4. Sample Handling & Labeling

Place all the rinsed material (or GF/F filter) into the centrifuge tube and wrap with foil to exclude light, write the Activity ID on the lid with a **SHARPIE.** Record the surface area of the template and the number of templates composited (this is a measure of standing crop so volume filtered is not necessary).

2.3. Hoop Method

The hoop method is designed for transects dominated by the presence of filamentous algae.

2.3.1. Method Summary

The hoop collection method is the collection of six sub-samples of uniform area from representative areas within the sampling site. The six (6) samples are composited into a single bag and submitted to the laboratory for extraction and analysis.

2.3.2. Sampling Equipment

- Waders
- Large freezer storage bags
- Aluminum foil
- Cooler
- Dry ice or regular ice
- Metal hoop (30 cm diameter, 710 cm^2 area)
- Scissors
- Tooth brush
- Knife for scraping rocks

2.3.3. Sample Collection

Select 6 locations in the reach that are representative of the algal densities observed. If a small number of macrophytes (< 5% by area) are present, they can be physically removed from the sample. If more are present, *do not use the hoop method*.

Regardless of stream substrate, a metal hoop with a 710 cm^2 area (30 cm diameter) is placed over the collection site.

All the algal material within the hoop is collected. Rocks within the hoop can be picked up and attached algae scraped into the freezer bag. Scissors can be used to detach the filamentous algae from their substrate.

Collect samples from the other 5 locations in the same manner.

2.3.4. Sample Handling & Labeling

Composite all filamentous algae collected from the six sites into a single (large) Ziploc freezer bag; place a label on the outside of the bag. Wrap the bag with foil leaving no space for light to enter. Place this wrapped bag into another large Ziploc bag and hand write the Activity ID on the outer bag with a **SHARPIE.**

Immediately store the sample on ice and away from light. Samples should be sent to the laboratory as soon as possible for chlorophyll-*a* analysis (monochromatic, corrected for pheophytin-a).

2.4. Core Method

Method for transects dominated by pools or by silt-clay substrate.

2.4.1. Method Summary

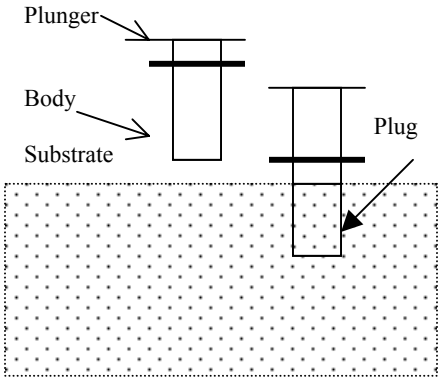
Three (3) core samples are taken from the substrate. The top 1cm of each core is sliced off the plug and placed in a centrifuge tube, resulting in a 3 core composite sample. The composite sample is sent into the laboratory for extraction & analysis.

2.4.2. Sampling Equipment

- Waders
- Cut-off 60 ml syringes (5.6cm^2)
- 50 ml centrifuge tubes
- Aluminum foil
- Small Ziploc bags
- Knife

2.4.3. Sample Collection

Three 5.6 cm^2 core samples are collected using a cut-off 60 cc syringe in a representative transect of the sampling site.



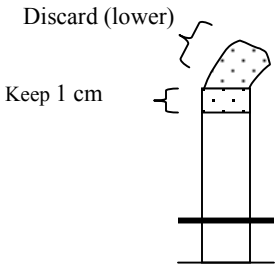
Each core sample is taken by driving the 60 cc syringe into the substrate to a depth of 5-10 cm. The syringe plunger may have to be drawn up as the body of the syringe sinks into the substrate to accommodate the core sample "plug". (The plunger may have too much friction within the barrel to rise on its own as the body of the syringe is pushed into the sediment)

The plug may be comprised of loose sediment that will fall out of the syringe. To minimize loss of a loose plug, the sampler may have to place a finger over the end of the syringe as it is pulled out of the hole and up through the water column.

In deeper water, a broom handle can be duck-taped to the syringe to extend your reach.

Immediately after the syringe containing the plug is out of the water, it must be inverted so that the plug does not slide out of the barrel.

The core is extruded so that the upper 1 cm of the core can be sliced off and placed in a 60 ml centrifuge tube. The remainder of the core sample is discarded.



Important: Only the **upper 1 cm** of each core sample is placed in a centrifuge tube. Assure that all the material adhering to the surface of the plunger-end is carefully collected, as most of the chlorophyll will be there.

A minimum of three (3) cores must be taken at the site for a representative sample.

2.4.4. Sample Handling & Labeling

Sampling location is identified on the centrifuge tube on an external label with the following information:

a) Sample Type
b) Activity ID
c) Collection Date
d) Waterbody Name
e) Collector's Name

After filling out the outside label, place it on the centrifuge tube, and cover it with clear tape.

Cover the centrifuge tube with aluminum foil, and place it in another small Ziploc bag. Handwrite the Activity ID on the outer bag with a **SHARPIE.**

Place samples in the cooler with ice.

2.5. Phytoplankton Method (chlorophyll-a in water)

The phytoplankton method is the sampling method for determining chlorophyll-a in the water column. It is used for transects dominated by pools with green color (light green to dark green) and lakes.

2.5.1. Method Summary

This method uses a filtration apparatus to collect a sample. Since chlorophyll-a breaks down readily in sunlight, the use of a dark Nalgene bottle is required to minimize the exposure of the sample to sunlight. The filter apparatus should be setup prior to sample collection to minimize the time between sampling and filtration. The volume of water filtered must be recorded!

2.5.2. Sampling Equipment

- 50 ml centrifuge tubes
- 1 - hand pump vacuum with tubing
- Nalgene filtering unit
- GFF filters (0.45 um)
- Tweezers or forceps

- Graduated cylinder (100-250 ml)
- Deionized water in squeeze bottle
- 1 L (Dark) Nalgene Bottle

2.5.3. Sample Collection

Filter apparatus setup

Using clean forceps, place a glass fiber filter (GF/F nominal pore size 0.7 um) on the filter holder. Use a small amount of deionized water from a wash bottle to help settle the filter properly.

Rinse the sides of the filter funnel and the filter with a small volume of deionized water.

Sample collection and processing

Rinse a 1L dark Nalgene bottle 3 times with stream or lake water before collecting the sample.

Grab a water sample using the 1L Nalgene bottle. Cap the bottle and thoroughly mix by inverting bottle 3 times.

Rinse a 100-250 ml. graduated cylinder three times with *deionized water*.

Measure 20 ml or more of sample in the graduated cylinder and pour it into the filter funnel, place the cap on the filter funnel, and draw the sample through the filter using the vacuum hand pump. **Note: vacuum from the pump should not exceed 9 inches of Hg on the gauge, to avoid rupture of fragile algal cells.**

Keep track of the volume of sample filtered! The volume of sample filtered can vary from 5 ml to 1000 ml or more. When filtration begins to slow and the filter has developed a distinct green (or green-brown) color, sufficient sample has been filtered. Do not allow the filter to get clogged, you cannot discard excess sample (water) from the filtration apparatus without losing a portion of the sample from the filter media (this also makes it difficult to keep track of volume filtered.). If a filter completely clogs while water remains in the upper half of the apparatus, you must discard the filter and start again, using less water volume.

After filtration is done, unplug the hand pump, remove the filter funnel from the filter holder, and remove the filter with clean forceps. Avoid touching the colored portion of the filter. Fold the filter in half, with the colored site folded in on itself. Place the folded filter paper in a 50 ml centrifuge tube.

2.5.4. Sample Handling & Labeling

Sample centrifuge tube should be identified with the following information:

- Sample Type
- Activity ID
- Collection Date
- Waterbody Name
- Collector's Name
- Volume filtered

Place the sample label on the centrifuge tube, and cover it with clear tape.

Cover the centrifuge tube with aluminum foil, and place it in another small Ziploc bag. Hand write the Activity ID on the outer bag with a **SHARPIE.**

Place samples in a cooler with ice. If possible, place samples on dry ice IF continuous freezing of the samples can be assured up to the time that they are delivered to the analytical laboratory.

3. Recording the chlorophyll-a sampling event

The chlorophyll-a sampling event must be recorded including information on the location, method used, and the number of samples collected and composited, or volume filtered. DEQ uses a Site Visit Form (SVF - Attachment 1) to record this information for later entry into the STORET database.

On the SVF, indicate a chlorophyll-a sample was taken by checking the chlorophyll-a box.

Note the Activity ID (Sample ID) on the Site Visit Form.

Circle the sample collection procedure on the Site Visit Form.

For **Rock** collection method circle **CHLPHL-2.**
For **Template** collection method circle **other** and write **CHLPHL-1**.
For **Hoop** collection method circle **HOOP**.
For **Core** collection method circle **CORE**.
For **phytoplankton** collection method circle **other** and write "**PHYTOPLANK"** next to it.

Complete the Chain of Custody Form. Include the numbers or volume of samples submitted so that the laboratory can complete their calculations to area or volume. For example:

For the **Rock** method, include the number of rocks collected on the comment section (e.g., 6 rocks).
For the **Template** method, include the surface area (in cm^2) of the template and the number of templates composited together, if any.
For the **Hoop** method, include the number of hoops composited and the area of each hoop (e.g., 6 hoops at 710 cm^2 each).
For the **Core** method, include the number of cores composited and the area of each core (e.g., 3 cores at 5.6 cm^2 each).
For the **phytoplankton** method, include the volume of water filtered (e.g., 1.25 L Filtered).

It is critical that complete records describing number of hoops or cores comprising *composite* samples are included in the documentation with the samples. Phytoplankton samples must include documentation of volume filtered. *The laboratory must not proceed until the field crew provides these documents.*

4. Sample Extraction

Sample extraction and Spectrophotometric determination are to be performed in an analytical laboratory by a qualified laboratory technician or chemist. Sample extraction and spectrophotometric determination are modified from the procedure described in EPA 446.0, "In Vitro Determination of Chlorophylls a, b, c_1 + c_2 and Pheopigments in Marine and Freshwater Algae by Visible Spectrophotometry, USEPA NERL-ORD, September 1997, Revision 1.2".

These modifications are: the exclusive use of the monochromatic equation for pheopigment-corrected chlorophyll-a and pheophytin-a, the allowance of methanol as the extraction solvent, and calculating the concentration of chlorophyll-a to an area for rock, hoop, template, and core sampling methods.

ALL CHLOROPHYLL WORK SHOULD BE PERFORMED IN SUBDUED LIGHT

If processing must be delayed, hold samples on ice or at 4^{o}C and protect from the exposure to light. Samples taken from water having a pH 7 or higher may be placed in airtight plastic freezer bags and stored frozen for 3 weeks. Samples from acidic water must be processed promptly to prevent chlorophyll degradation.

The five different sampling techniques result in different types of media being submitted to the laboratory, extractions to accommodate each media follow.

4.1. Rock samples

This extraction must be performed in subdued light to minimize the degradation of chlorophyll-a pigment. A rock sample should consist of 6 rocks. Any differences should be noted on the chain-of-custody when the samples arrive. They should arrive at the laboratory in a zip lock bag covered with aluminum foil, which is in a second (outer) zip lock bag. Samples should arrive frozen and remain frozen until ready for analysis.

4.1.1. Sample Extraction

- When ready to extract, remove sample from freezer and allow it to warm up.
- Quantitatively transfer rocks to a 3-gallon whirl-pak bag.
- Add enough methanol to cover the rocks.
- Check bag for leaks and record the extract volume.
- Seal and label the bag and keep in the dark overnight.
- The next day, proceed with spectrophotometric analysis (Part 5 of this SOP)

4.2. Hoop Samples

This extraction must be performed in subdued light to minimize the degradation of chlorophyll-a pigment. A hoop sample should be a composite of all periphyton algae taken from six 710 cm^2 areas. If it is not clear on the chain-of-custody how many samples were composited, *contact the sampler before proceeding.* Samples should arrive at the laboratory in a zip lock back covered with aluminum foil, and with a second (outer) zip lock bag protecting the aluminum foil. Samples should be frozen upon arrival and remain frozen until ready for analysis.

4.2.1. Sample Extraction

- When ready to extract, remove sample from freezer and allow it to thaw.
- Remove any excess water by straining or decanting. Excessive water interferes with the spectrophotometric measurement.
- Add enough methanol to the (inner) zip lock bag to cover the sample. Record volume added. A minimum of 13 ml of methanol is needed for analysis. Additional methanol may be added, however the more methanol added, the greater the dilution of pigments – *don't dilute yourself into a non-detect.*
- Identify the sample with a label.
- Keep in the dark overnight.
- The next day, proceed with spectrophotometric analysis (Part 5 of this SOP)

4.3. Core Samples

This extraction must be performed in subdued light to minimize the degradation of chlorophyll-a pigment. A core sample should be a composite of the *upper 1 cm* of three 5.6 cm^2 core plugs. The composite sample should be returned from the field in a 50 ml centrifuge tube wrapped in aluminum foil. This foil wrapped tube should be in a protective (outer) zip lock bag. Sample should arrive frozen and remain frozen until ready for extraction.

4.3.1. Sample Extraction

- When ready to extract, remove sample from freezer and allow it to thaw.
- Add enough methanol to the centrifuge tube to cover the sample. Record volume added. A minimum of 13 ml of methanol is needed for analysis. Additional methanol may be added, however the more methanol added, the greater the dilution of pigments – *don't dilute yourself into a non-detect.*
- Core samples can be very thick and compact. It may be necessary to mix the methanol and sample using a mechanical shaker.
- Keep in the dark overnight.
- The next day, proceed with spectrophotometric analysis (Part 5 of this SOP)

4.4. Template Samples

This extraction must be performed in subdued light to minimize the degradation of chlorophyll-a pigment. Template samples may arrive at the laboratory as either a frozen water sample or as a frozen filter sample depending on the equipment available to the sampling crew. Because of this, template samples must be clearly identified on the chain-of-custody as such so that they are not confused with phytoplankton samples. Template samples are scrapings and subsequent washing of equipment with water that are subsequently filtered and the filter extracted. Field crews may have filtration equipment available and return samples (on filters) in centrifuge tubes. Regardless, the template area must be included on the chain-of-custody prior to proceeding with the analysis because results are reported as mass/area. Samples should be returned to the laboratory frozen and remain frozen until analysis.

4.4.1. Sample Extraction

- Assemble filtration apparatus and quantitatively transfer and filter entire sample through a 47 mm glass fiber filter with a nominal pore size of 0.7 um. (Whatman GF/F filters)
- Volume filtered is irrelevant because results will be related to template area.
- Place filter into a labeled centrifuge tube.
- Add a minimum of 13 ml. of methanol to centrifuge tube and keep in the dark overnight. Do not add more than 20 ml of methanol unless multiple filters were required for the sample. Make sure the entire filter is covered by methanol. Record volume of methanol added.
- Keep sample in the dark overnight.
- The next day, proceed with spectrophotometric analysis (Part 5 of this SOP)

4.5. Phytoplankton Samples

This extraction must be performed in subdued light to minimize the degradation of chlorophyll-a pigment. Phytoplankton samples may arrive at the laboratory as either a water sample or as a filter in an aluminum foil wrapped centrifuge tube. Filtering of water samples should be performed as soon as samples arrive since algal populations can change in a relatively short period of time. Filtration in the field is preferred.

4.5.1. Sample Extraction

Filter apparatus setup

- Using clean forceps, place a glass fiber filter (0.45 um) on the filter holder. Use a small amount of deionized water from a wash bottle to help settle the filter properly.
- Rinse the sides of the filter funnel and the filter with a small volume of deionized water.
- Cap the bottle and thoroughly mix by inverting bottle 3 times.
- Rinse a 100-250 ml. graduated cylinder three times with *deionized water*.
- Measure 20 ml or more of sample in the graduated cylinder and pour it into the filter funnel, place the cap on the filter funnel, and draw the sample through the filter using the vacuum hand pump. **Note: vacuum from the pump should not exceed 9 inches of Hg on the gauge, to avoid rupture of fragile algal cells.**
- **Keep track of the volume of sample filtered!** The volume of sample filtered can vary from 5 ml to 1000 ml or more. When filtration begins to slow and the filter has developed a distinct green (or green-brown) color, sufficient sample has been filtered. Do not allow the filter to get clogged, you cannot discard excess sample (water) from the filtration apparatus without losing a portion of the sample from the filter media (this also makes it difficult to keep track of volume filtered.). If a filter completely clogs while water remains in the upper half of the apparatus, you must discard the filter and start again, using less water volume.
- After filtration is done, unplug the vacuum pump, remove the filter funnel from the filter holder, and remove the filter with clean forceps. Avoid touching the colored portion of the filter. Fold the filter in half, with the colored site folded in on itself. Place the folded filter paper in a 50 ml centrifuge tube.

5. Spectrophotometric Determination of Chlorophyll-a

The Spectrophotometric determination of chlorophyll-a is to be performed according to EPA Method 446.0 or Standard Methods 10200 H. Chlorophyll, 20th edition or later. Both of these methods include the monochromatic calculation required by DEQ for pheophytin-a correction [as per ARM 17.30.602 Definitions (5)].

Each laboratory must have current standard operating procedures (SOPs) that describe their instruments, reagents, interferences, standards, instrument set-up, calibration procedures, analytical procedures, quality control requirements, calculations, and reporting protocols. Except as provided below, these SOPs must describe a method in general accordance with the reference methods (EPA 446.0 and Std. Meth. 10200H).

5.1. Correction for pheophytin-a

Both reference methods (EPA 446.0 and Standard Methods 10200H) provide calculations for obtaining monochromatic (chlorophyll-a corrected for presence of pheophytin-a) and trichromatic (chlorophyll-a,b,c) results. The data used to develop the Water Quality Standards for the Clark Fork River (sec. 1.3.1), DEQ's Hi-line study, and reference conditions used the monochromatic calculations. Therefore, ALL chlorophyll-a results intended for beneficial use support determinations must use the monochromatic calculation.

Refer to Standard Methods 10200H(2) or EPA Method 446.0, Section 12.2 for instrument requirements, sample analysis requirements (calibrations, reagents, wavelengths, and calculations). The calculations presented in the reference methods are for a phytoplankton (water) sample and can be applied directly for those samples.

Samples submitted for measurement of periphyton chlorophyll a must be calculated based on area. Generally, the area of the sample (rather than the volume of water filtered) divides the mass of chlorophyll-a determined from the monochromatic calculation.

The calculation for periphyton should be obtained from the DPHHS Laboratory Director, Judy Halm (444-5259) or the DEQ QA Officer, Mark Bostrom (444-2680).

5.2. Calculation to area

In order to determine the density of periphyton algae by measuring chlorophyll-a, results obtained from the instrument (in mg) must be related to the area sampled rather than a volume of water. The area obtained from the various collection techniques varies.

5.2.1. Area of Rocks (post analysis)

- Area of the sample is determined by carefully wrapping aluminum foil around *each* rock and trimming off the excess foil. This foil is removed and weighed. Area is determined by comparing the mass of the foil(s) from all rocks against a calibration curve consisting of known areas that have been weighed and plotted to create a calibration curve.
- Creating calibration curve for determining area. The following sizes are recommended for the aluminum foil mass-to-area curve (R^2 linear regression).
  - 0
  - 100 cm^2
  - 400 cm^2
  - 1600 cm^2
- Curves with more than four points (three measurements set through zero) do not substantially improve the curve but have been used by some laboratories.
- Once the total area is established, it is divided by two to approximate the area of the rock that would be considered substrate (facing up - subject to periphyton growth), and exclude the portion that is embedded (facing down).

5.2.2. Area of Hoops

- Each hoop has a standard area of 710 cm^2. Six hoops are required for a representative sampling. The number of hoop samples composited and submitted to the laboratory must be confirmed from the chain-of-custody. Calculate as follows:

$$\frac{710\ cm^2 \times 6\ "hoops"}{10{,}000\ cm^2 / m^2} = 0.426\ m^2$$

5.2.3. Area of Cores

- Each core has a standard area of 5.6 cm^2. Three cores are required for a representative sampling. The number of cores composited and submitted to the laboratory must be confirmed from the chain-of-custody. Calculate as follows:

$$\frac{5.7\ cm^2 \times 3\ "cores"}{10{,}000\ cm^2 / m^2} = 0.0017\ m^2$$

5.2.4. Area of Templates

- Templates may vary in size and number of samplings composited. The number of composites and standard size of the template must be submitted on the chain-of-custody. The

calculation then becomes a matter of summing the area of all composites and converting to square meters.

References

American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998. Method 10200 H. Chlorophyll.

US Environmental Protection Agency, National Exposure Research Laboratory, Office of Research and Development, Method 446.0, *In Vitro* Determination of Chlorophylls a, b. c_1, $+c_2$ and Pheopigments in Marine And Freshwater Algae by Visible Spectrophotometry. Adapted by Elizabeth J. Arar, Revision 1.2, September 1997

Montana Department of Public Health and Human Services Environmental Laboratory, Standard Operating Procedure, EPA 446.0 Modified Chlorophyll. Revision 1, 01/08/04, Prepared by Lee Harbour

ATTACHMENT 1

SOP WQPBWQM-011
DEQ SITE VISIT FORM

Place Site Visit Label Here

Site Visit Form

(One Station per page)

STORET Project ID:_____

Trip ID :_____

Date _____ Personnel _____

Waterbody _____ Location _____

Station ID _____ Visit # _____ HUC _____ County _____

Lat _____ Long _____ GPS Datum (Circle One): NAD 27 NAD 83 WGS84 Lat/Long Verified? ☐ By _____

Lat/Long obtained by method other than GPS? Y ☐ N ☐ If Y what method used? If by map, provide map scale _____

Samples Taken:			**Sample ID** (Provide for **all** samples)	**Sample Collection Procedure**
Water	☐	Nutrients ☐ Metals ☐ Commons ☐		GRAB
Sediment	☐			SED-1
Chlorophyll a	☐			CHLPHL-2 HOOP CORE OTHER:
Algae/Macrophytes	☐			PERI-1 OTHER:
Macroinvertebrate	☐	Macroinvertebrate Habitat Asmt. ☐		KICK HESS JAB OTHER:

Kick/Jab length (ft):	Kick Duration / # Jabs:	No of Jars:	Mesh Size: 1000 500 OTHER:

Habitat Assessment	☐	Stream Reach Asmt. ☐ Other ☐
Substrate	☐	Pbl. Count ☐ % Fines ☐ RSI ☐
Channel X-Section	☐	
Photographs	☐	
Flow - Measured	☐	
Other:		

Measurements:	**Time:**	
Temp: (˚C)	W	A °C °F
pH:		
SC: (mS/cm)		
SC: (mS/cm) x 1000		µmho/cm
DO: (mg/L)		
Flow Estimated:		cfs V☐ F ☐
Flow Comments:		
TUR: Clear ☐ Slight ☐ Turbid ☐ Opaque ☐		
Turbidity Comments:		

Meter Number:
Site Visit Comments: